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Photoperiod-specific LED Supplementation Enhances Growth Performance and Alters Biomass Allocation in Mustard Greens (*Brassica Juncea* L.) under Controlled Environment Agriculture

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ABSTRACT

Optimising supplemental lighting protocols for tropical controlled environment agriculture remains challenging due to limited photoperiod-specific research. This study investigated LED timing effects on mustard greens (*Brassica juncea* L.) growth under Vietnamese greenhouse conditions. Four treatments were evaluated: LED_0 (control), LED_18-22 pm (evening supplementation), LED_22 pm - 2 am (night supplementation), and LED_2-6 am (early morning supplementation). Custom LED modules with 50W Chip-on-Board technology provided 60-70 µmol.m^{-2}.s^{-1} PPFD at 0.5m distance. Plants were grown in commercial Tribat soil with automated irrigation maintaining 70-80% moisture. Morphological parameters, SPAD chlorophyll index, and biomass allocation were assessed over 42 days. Evening LED supplementation (18-22 pm) demonstrated superior performance, achieving a 70.7% increase in plant height (61.64 vs 36.10 cm), a 58.0% enhancement in total fresh weight (20.65 vs 13.07 g), and a 26.2% improvement in leaf number compared to controls. However, this treatment exhibited morphological trade-offs with a 11.8% reduction in leaf width and 18.2% decrease in leaf length. Biomass allocation shifted dramatically from 90.15% leaf allocation in controls to 77.06% under evening supplementation, with stem allocation increasing from 1.92% to 17.33%. SPAD analysis revealed timing-dependent chlorophyll responses, with early enhancement under evening treatment and superior mid-season development under early morning supplementation. These findings establish that 4-hour evening LED supplementation optimally synchronises with *Brassica juncea* circadian

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rhythms, providing an energy-efficient enhancement of productivity while highlighting critical morphological consequences for commercial applications in tropical controlled-environment agriculture.

Keywords: Brassica juncea, biomass allocation, controlled environment agriculture, LED timing, photoperiod optimisation, tropical greenhouse

INTRODUCTION

With rapid urbanisation and growing food security demands, controlled environment agriculture (CEA) has emerged as a critical solution for sustainable crop production, particularly where arable land is limited (Acero, 2013; Rouphael et al., 2010). Indoor farming systems utilising artificial lighting enable year-round production of high-quality crops with minimal environmental impact (Bian et al., 2015; Dou et al., 2018). However, low natural daily light integrals (DLIs) during darker months, particularly in higher-latitude regions (5-15 $mol \cdot m^{-2} \cdot d^{-1}$), and seasonal limitations in tropical regions during rainy seasons necessitate optimised supplemental lighting systems (Faunce, 2012; Pham et al., 2024).

Light-emitting diodes (LEDs) have revolutionised agricultural lighting by offering superior energy efficiency, precise wavelength control, and reduced heat generation compared to traditional systems (Cocetta et al., 2017; Mitchell et al., 1991). The spectral tunability of LEDs enables targeted manipulation of photoreceptor systems, particularly through red (660-670 nm) and blue (440-460 nm) wavelengths that demonstrate high photosynthetic efficiency (Massa et al., 2008; Olle & Viršile, 2013). Photosynthesis is highly dependent on photosynthetically active radiation (PAR, 400-700 nm), with blue light crucial for chlorophyll formation and stomatal regulation, while red light efficiently drives photosynthetic energy conversion and biomass accumulation (Acero, 2013; Terashima et al., 2009).

Brassica species, including leafy Brassica vegetables, are particularly valuable due to their rapid growth cycles, high responsiveness to light quality modifications, and complex secondary metabolite pathways contributing to health-promoting properties (Kyriacou et al., 2016). These crops demonstrate optimal growth under controlled conditions with PPFD ranges of 220-440 $\mu mol.m^{-2}.s^{-1}$, making them ideal candidates for LED-based production systems (Jones-Baumgardt et al., 2019; Samuolienė et al., 2013).

Recent research has expanded beyond traditional red-blue LED combinations to explore effects of broader spectral ranges, with studies showing that light intensity significantly influences morphology and biomass accumulation (Brazaitytė et al., 2019; Gerovac et al., 2016). However, significant knowledge gaps remain regarding optimal LED strategies for enhancing both productivity and phytochemical content in leafy Brassica crops, particularly under practical greenhouse conditions (Podsędek et al., 2023). Despite lighting costs accounting for 40-80% of total production expenses, comprehensive investigations of spectral effects on primary metabolites, carotenoids, chlorophylls, and antioxidant compounds remain limited (Podsędek et al., 2023; Semenova et al., 2022).

Based on 28 recently different collected studies, it shows that only 3 experiments were conducted in temperate regions, with the majority being conducted in temperate and subtropical regions on seedlings (microgreens) deployed in strictly controlled environments such as growth chambers or laboratories, where light, temperature and humidity are artificially regulated, commonly using LED lights or other artificial light sources. Supplemental experiments with LED lighting at different times throughout the night when grown on clean soil under greenhouse conditions have not been found. The critical knowledge gap in photoperiod-specific LED supplementation for tropical microgreen production under practical greenhouse conditions necessitates this investigation. While existing studies predominantly focus on controlled laboratory environments in temperate regions, the unique challenges of tropical agriculture, including seasonal variations in natural light intensity, high humidity conditions, and economic constraints of small-scale farmers, remain inadequately addressed. Furthermore, the optimisation of LED timing protocols specifically for *Brassica juncea* cultivars adapted to Southeast Asian growing conditions has not been systematically evaluated, despite the species' significant commercial potential and nutritional value in regional markets. The substantial economic implications of lighting costs (40-80% of production expenses) combined with limited understanding of circadian rhythm synchronisation under tropical photoperiods create an urgent need for evidence-based LED supplementation strategies. Recent studies have begun to address this photoperiod-timing gap directly. A hydroponic trial comparing the same 4-hour supplemental windows (18:00-22:00, 22:00-02:00, and 02:00-06:00) in mustard greens found that pre-dawn (02:00-06:00) lighting produced the greatest gains in SPAD index, leaf number, plant height, and leaf width, yielding 44% higher fresh weight and 52% higher dry biomass than the control, with the highest leaf-to-total biomass ratio and lowest stem ratio (Duong et al., 2026). Separately, a photoperiod-duration trial (8-24 hours) on *Brassica* microgreens showed that short photoperiods (8 hours) promoted hypocotyl elongation and fresh weight, whereas continuous 24-hour illumination suppressed growth most severely (Vaštakaitė-Kairienė et al., 2022). These findings, however, were obtained in hydroponic or short-cycle microgreen systems rather than in soil-grown mustard greens over a complete production cycle, leaving the comparative efficacy of specific nocturnal timing windows under tropical greenhouse soil culture still unresolved. Therefore, this study aims to bridge these critical gaps by evaluating photoperiod-specific LED timing effects on growth performance, biomass allocation, and physiological responses of mustard greens under realistic Vietnamese greenhouse conditions, providing essential relevance for sustainable intensification of tropical controlled environment agriculture systems in the future.

MATERIALS AND METHODS

Experimental Conditions

The greenhouse experiment was conducted at Kien Giang University, Chau Thanh district, Kien Giang province, Vietnam (9°54'49.05"N, 105°8'33.7992"E) from March 3rd to April 14th, 2025. Environmental parameters were continuously monitored using calibrated instruments: an Indoor Digital Temperature Humidity Meter (Beurer, Type: HM 16, Art.-Nr: 679.15) for air humidity and temperature measurements, and a Photosynthesis Light Quantum Meter (TES 1339P) for recording Photosynthetic Photon Flux Density (PPFD). The mean environmental conditions inside and outside the greenhouse were recorded as follows: PPFD (208/750 $\mu mol.m^{-2}.s^{-1}$), temperature (30/32 °C), and humidity (71/61%), respectively.

Growth Medium and Lighting System

Growth Medium

The growing substrate consisted of commercially available clean soil (Tribat soil, Sai Gon Xanh company, Vietnam) specifically formulated to provide adequate nutrition for mustard cultivation. The soil was filled to a depth of 2 dm in containers measuring 1 m width × 10 m length. The nutrient composition of Tribat soil (dry weight basis) included: humus (15%), organic matter (25%), total nitrogen (1%), potassium K_2O (0.73%), total phosphate P_2O_5 (0.3%), cation exchange capacity (CEC: 45 $meq.100g^{-1}$), pH 6.5, and salinity 1298 ppm. Additional micronutrients such as Mg, Mn, Zn, B, Cu, Mo, and chelated iron (Fe-EDTA-13) were present, though specific concentrations were not disclosed by the manufacturer.

LED Lighting System

A custom LED module was designed and assembled by the research team based on preliminary trials. Each module comprised four Chip-on-Board (CoB) LEDs arranged with one pink light centrally positioned and three white lights surrounding it (Figure 1, left and middle), with power consumption about 800Wh. All commercially available generic full-

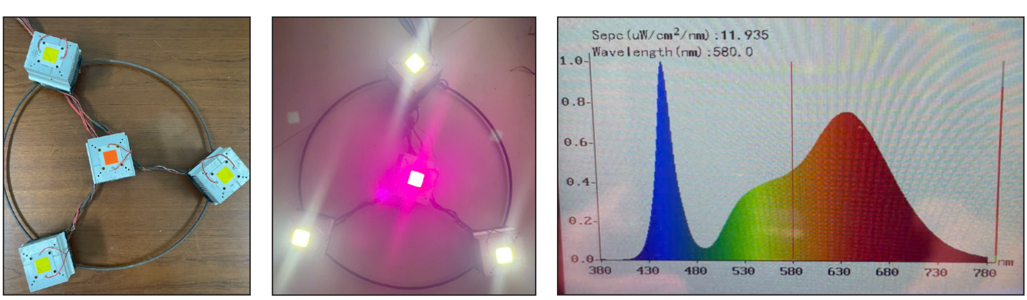

Figure 1. LED module: off (left), on (middle) and its spectral quality (right)

spectrum COB LED chips (46 × 40 mm package, model code 4640-4C30B) were sourced from China and shared identical specifications: voltage 12V-14V DC, power consumption 50 watts, luminous efficacy 90-120 LM/W, operating temperature range -20 °C to 60 °C, and spectral bandwidth 400-780 nm. COB modules were selected for their low cost, local availability, and ease of custom module fabrication for a multi-treatment timing experiment, which would have been cost-prohibitive with dedicated horticultural LED arrays.

Spectral quality was quantified using an OHSP-350BF Portable Spectral Irradiance Colourimeter (wavelength range: 380-780 nm), with an R:G:B ratio of 48:32:20 (Figure 1, right). Photosynthetic photon flux density (PPFD) of 60-70 µmol.m^{-2}.s^{-1} was measured using a Photosynthesis Light Quantum Meter (TES-1339P) at a distance of 0.5 m from the centre of the LED module. The spacing between COB LEDs was 40.05 cm (white-to-white) and 23 cm (white-to-pink).

Experimental Design

The experiment followed a completely randomised design with three replications conducted under controlled greenhouse conditions. Four distinct LED lighting treatments were evaluated: LED_0 (control treatment with LED system off), LED_18-22 pm (LED illumination from 6:00 PM to 10:00 PM), LED_22 pm-2 am (LED illumination from 10:00 PM to 2:00 AM), and LED_2-6 am (LED illumination from 2:00 AM to 6:00 AM). Each treatment consisted of 3 pots with dimensions of 57 cm (length) × 36 cm (width) × 16 cm (height), resulting in a total of 12 experimental plots.

The LED modules were positioned to maintain a consistent distance of 0.5 m from the lamp to the leaf tips, as determined through pilot studies; the LED module height was adjusted (raised) as the plants grew. Supplemental lighting was initiated when mustard seedlings reached 7 days after germination. The on/off cycling of the LED modules for each treatment was automatically controlled using a digital timer switch (ATA, Model AT-20B). This programmable timer allowed multiple independent on/off switching cycles to be preset within a 24-hour period, with a minimum switching interval of 1 minute. Prior to the experiment, each timer was programmed and calibrated according to the designated photoperiod schedule for each treatment (LED_18-22h, LED_22h-2h, and LED_2-6h), ensuring that the LED modules were automatically activated and deactivated at the specified start and end times daily throughout the growth period. The control treatment (LED_0) was connected to the power supply but remained switched off for the entire experimental duration (Figure 2). Each timer unit was connected in series between the main power source and the corresponding LED module, and its accuracy was periodically verified against a reference clock to ensure consistency of the photoperiod treatments across.

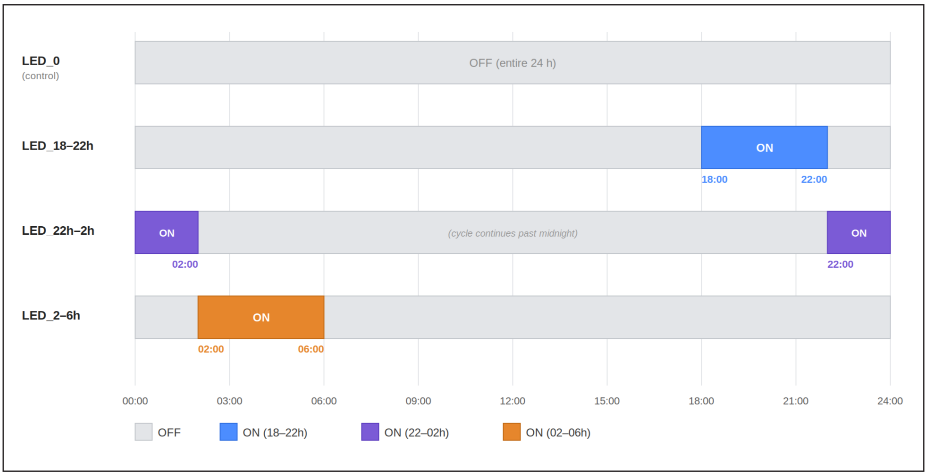

Figure 2. 24-hour LED on/off Schedule by treatment

Crop Establishment and Management

Seed Establishment

Mustard green seeds obtained from Vietnamese Dai Dia company, representing a tropical variety commonly cultivated in southern Vietnam, were directly sown onto the Tribat soil substrate at a seeding rate of 0.2 g per square meter. Soil moisture was maintained consistently at 70-80% throughout the experimental period using a garden hose irrigation system, with moisture levels monitored using a soil moisture tester (MD-15). Non-uniform seedlings were removed to achieve and maintain a uniform population density of 100 plants per square meter.

Data Collection and Measurements

Growth Parameters and Yield Assessment

Initial measurements commenced when mustard plants developed three fully expanded leaves (one week after light treatment initiation), followed by subsequent measurements at 5-day intervals. From each plot, 45 randomly selected and marked plants (15 plants per pot) were used for growth parameter collection. Morphological measurements included leaf length (measured from the tip of the longest leaf to the petiole), leaf width (at the widest point), and plant height (from ground level to the tip of the topmost leaf).

Yield assessment was conducted 42 days after seeding by flooding the pots to facilitate soil-root separation. Plant components (leaves, stems, and roots) were carefully separated and weighed to determine fresh yield parameters.

SPAD Index Measurement

Chlorophyll content was estimated using the SPAD-502Plus Chlorophyll Meter. The third fully expanded leaf from the top was randomly selected from ten plants in each plot. The final SPAD value represented the mean of 15 individual readings per plot.

Growth Rate Calculation

Absolute Growth Rate (AGR) was calculated using the following Equation 1 (Lalruatfeli, 2021):

$$AGR = (W_2 - W_1) / (T_2 - T_1) \ (g.day^{-1}) \quad [1]$$

where W_1 and W_2 represent dry matter weight at times T_1 and T_2, respectively.

Statistical Analysis

Data analysis was performed using Analysis of Variance (ANOVA) appropriate for a split-plot experimental design. Mean separation was conducted using Duncan's Multiple Range Test at a $P \leq 0.05$ significance level. All statistical analyses were executed using SPSS Version 20.0 software package.

RESULTS

Effect Of Various LED Lighting Timings on Mustard Green Growth

Based on the experimental data, LED supplemental lighting significantly enhanced mustard green growth compared to the control treatment. The LED_18-22 pm treatment demonstrated superior performance, achieving the highest final plant height of 61.64 cm at 42 days after sowing (DAS), representing a 70.7% increase over the control (36.10 cm) (Table 1). This treatment maintained consistent growth throughout the experimental period, with notable acceleration during periods 2-4. The LED_2-6 am treatment produced intermediate results (45.81 cm final height), while LED_22 pm-2 am showed the poorest performance among supplemental lighting treatments (32.85 cm). Growth rate analysis revealed that the LED_18-22 pm treatment exhibited the most balanced growth pattern, with reduced initial growth rate (1.02 $cm \cdot day^{-1}$) but sustained performance in later periods. These findings suggest that evening LED supplementation (18-22 pm) optimally synchronises with the natural photoperiod and circadian rhythms of *Brassica juncea*, resulting in enhanced vegetative growth compared to other timing regimens or natural lighting conditions alone.

LED supplemental lighting exhibited contrasting effects on leaf width development in mustard greens compared to plant height responses. The control treatment (LED_0) achieved the greatest final leaf width of 8.28 cm at 42 DAS, significantly outperforming

Table 1
*Effect of various LED lighting timings on plant height (cm) of mustard greens (*Brassica juncea *L.)*

Treatment	Mean Plant Height (cm)					Height Growth Rate ($cm.day^{-1}$)			
	14 DAS	**21 DAS**	**28 DAS**	**35 DAS**	**42 DAS**	**Period1**	**Period2**	**Period3**	**Period4**
LED_0	11.04b	21.37b	25.04b	31.10c	36.10c	1.08a	1.95a	1.41a	1.48a
LED_18-22 pm	15.49a	21.27b	36.10a	48.27a	61.64a	1.02a	0.82b	0.80b	1.00b
LED_22 pm-2 am	15.06a	22.42a	26.64b	29.52c	32.85c	1.11a	1.13b	0.85b	0.80b
LED_2-6 am	16.17a	23.90a	33.76a	40.01b	45.81b	0.60b	1.16b	0.61b	0.61b
Sig.	***	***	***	***	***	**	***	***	***

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am, and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: ** ($P < 0.01$), *** ($P < 0.001$). Days after sowing: DAS the LED_18-22 pm treatment (7.30 cm), which showed a 11.8% reduction (Table 2). Early growth patterns revealed that LED_18-22 pm initially promoted leaf width expansion at 14 DAS (4.15 cm vs. 3.35 cm in control), but this advantage diminished over time, with slower growth rates observed in periods 2-4 (0.10, 0.06, and 0.09 $cm \cdot day^{-1}$, respectively). The LED_22 pm-2 am treatment demonstrated the highest leaf width at 28 DAS (7.16 cm) with the most rapid expansion during period 2 (0.26 $cm \cdot day^{-1}$), though data beyond this timepoint were unavailable. These findings suggest that while evening LED supplementation (18-22 pm) effectively promotes vertical growth, it may redirect metabolic resources away from lateral leaf expansion, potentially indicating a trade-off between height and leaf width development under artificial photoperiod manipulation in *Brassica juncea*.

LED supplemental lighting significantly influenced leaf length development in mustard greens, with the control treatment maintaining superior performance throughout the experimental period (Table 3). The LED_0 treatment achieved the highest final leaf length of 21.78 cm at 42 DAS, while LED_18-22 pm showed the poorest response with only 17.81 cm, representing an 18.2% reduction compared to the control. Despite initial advantages in early growth (11.33 cm at 14 DAS vs. 9.47 cm in the control), the LED_18-22 pm treatment exhibited dramatically reduced growth rates in later periods (0.20, 0.09, and 0.09 $cm \cdot day^{-1}$ for periods 2-4, respectively), compared to the control's consistent rates (0.47, 0.24, and 0.24 $cm \cdot day^{-1}$). The LED_22 pm-2 am treatment performed comparably to the control (21.44 cm final length) with the highest growth rate during period 2 (0.58 $cm \cdot day^{-1}$). These results demonstrate that evening LED supplementation (18-22 pm) creates a pronounced trade-off between vertical stem elongation and leaf length expansion, suggesting that while this photoperiod manipulation effectively promotes plant height, it simultaneously constrains leaf morphological development in *Brassica juncea*.

LED supplemental lighting significantly enhanced leaf number development in mustard greens, with LED_18-22 pm and LED_2-6 am treatments demonstrating superior performance compared to the control (Table 4). The LED_18-22 pm treatment achieved the highest leaf number at 35 and 42 DAS (10.289 and 10.378 leaves, respectively), representing a 26.2% and 20.0% increase over the control (8.156 and 8.644 leaves). The LED_2-6 am treatment produced the greatest final leaf count of 10.667 leaves at 42 DAS, showing a 23.4% improvement compared to the control. Notably, LED_18-22 pm exhibited an exceptional leaf production burst during period 2 (0.53 $leaves \cdot day^{-1}$), the highest rate among all treatments and periods, followed by dramatic reductions in later periods (0.03 and 0.01 $leaves \cdot day^{-1}$). The LED_2-6 am treatment demonstrated the most consistent leaf development pattern with sustained growth rates throughout the experimental period (0.39, 0.11, and 0.17 $leaves \cdot day^{-1}$ for periods 2-4). These findings indicate that both evening (18-22 pm) and early morning (2-6 am) LED supplementation effectively stimulate leaf initiation and development, with evening lighting promoting rapid early leaf emergence while early morning lighting maintains more sustained leaf production in *Brassica juncea*.

Table 2
*Effect of various LED lighting timings on leaf width of mustard greens (*Brassica juncea *L.)*

Treatment	Mean Leaf Width (cm)					Leaf Width Growth Rate (cm)			
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS	Period1	Period2	Period3	Period4
LED_0	3.35c	5.47b	6.79 b	7.49a	8.28a	0.30a	0.19b	0.10a	0.11a
LED_18-22 pm	4.15a	5.59b	6.27c	6.69b	7.30b	0.21b	0.10c	0.06b	0.09b
LED_22 pm-2 am	3.69b	5.34b	7.16 a	-	-	0.24b	0.26a	-	-
LED_2-6 am	3.82b	5.86a	6.87 ab	-	-	0.29a	0.14bc	-	-
Sig.	***	**	***	***	***	***	***	***	*

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2am and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$). Days after sowing: DAS. "-" indicates data missing due to a field data-recording oversight

Table 3
*Effect of various LED lighting timings on leaf length of mustard greens (*Brassica juncea *L.)*

Treatment	Mean Leaf Length (cm)					Leaf Length Growth Rate ($cm.day^{-1}$)			
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS	Period1	Period2	Period3	Period4
LED_0	9.47b	15.13	18.44a	20.12a	21.78a	0.81	0.47a	0.24a	0.24a
LED_18-22 pm	11.33a	15.16	16.56b	17.21c	17.81c	0.55	0.20c	0.09c	0.09c
LED_22 pm-2 am	10.70a	14.83	18.92a	20.04a	21.44a	0.59	0.58a	0.16b	0.20ab
LED_2-6 am	11.00a	18.27	17.94a	19.02b	20.27b	0.66	0.33b	0.15b	0.18b
Sig.	***	ns	***	***	***	**	***	***	***

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am and 2- 6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: ** ($P < 0.01$), *** ($P < 0.001$), ns (not significant). Days after sowing: DAS

Table 4
*Effect of various LED lighting timings on leaf number of mustard greens (*Brassica juncea *L.)*

Treatment	Mean Leaf Number					Leaf Number Growth Rate (Leaf.day^{-1})			
	14 DAS	**21 DAS**	**28 DAS**	**35 DAS**	**42 DAS**	**Period1**	**Period2**	**Period3**	**Period4**
LED_0	4.93b	6.42a	7.24d	8.156c	8.644b	0.21a	0.12c	0.13a	0.07b
LED_18-22 pm	4.89b	6.40a	10.11a	10.289a	10.378a	0.22a	0.53a	0.03b	0.01c
LED_22 pm-2 am	4.78b	5.56c	7.80c	8.178c	8.489b	0.11b	0.32b	0.05b	0.04bc
LED_2-6 am	5.60a	5.98b	8.69b	9.489b	10.667a	0.05b	0.39b	0.11a	0.17a
Sig.	***	***	***	***	***	***	***	***	***

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am, and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: *** ($P < 0.001$). Days after sowing: DAS

Effect of Various LED Lighting Timings on SPAD Index of Mustard Green

LED supplemental lighting demonstrated significant effects on chlorophyll content (SPAD index) in mustard greens during early and mid-growth stages. The LED_18-22 pm treatment exhibited the highest SPAD value at 14 DAS (30.07), representing a 12.1% increase over the control (26.83), indicating enhanced early chlorophyll synthesis under evening LED supplementation (Table 5). However, the most pronounced response occurred with LED_2-6 am treatment at 35 DAS, achieving a SPAD value of 35.83, which was significantly higher than all other treatments and represented a 13.9% increase compared to the control (31.45). This delayed chlorophyll enhancement suggests that early morning LED supplementation optimally synchronises with natural photosynthetic processes during the critical reproductive transition period. Interestingly, SPAD differences among treatments became non-significant at the final harvest (42 DAS), with all treatments converging to similar chlorophyll levels (32.14-33.24). These findings indicate that LED photoperiod manipulation primarily influences chlorophyll development timing rather than final chlorophyll accumulation, with early morning supplementation (2-6 am) providing the most substantial mid-season chlorophyll enhancement in *Brassica juncea*.

Effect of Various LED Lighting Timings on Yield and Biomass Allocation of Mustard Green Growth

LED supplemental lighting significantly enhanced total biomass production and altered biomass allocation patterns in mustard greens (Table 6). The LED_18-22 pm treatment achieved the highest total fresh weight of 20.65 g, representing a 58.0% increase over the control (13.07 g), primarily attributed to superior leaf (15.68 g vs. 11.74 g) and stem development (4.19 g vs. 0.26 g). This treatment demonstrated a dramatic shift in biomass allocation, with stem biomass allocation increasing from 1.92% in the control to 17.33%, while leaf allocation decreased from 90.15% to 77.06%. The LED_22 pm-2 am and LED_2-6 am treatments showed even more pronounced stem allocation increases (20.94% and 21.73%, respectively) but with reduced total biomass production. Root biomass allocation significantly decreased under LED supplementation, particularly in the LED_2-6 am treatment (6.54% vs. 9.22% in control). These findings indicate that evening LED supplementation (18-22 pm) optimally balances enhanced total biomass production with modified architectural development, promoting stem elongation while maintaining substantial leaf biomass for photosynthetic capacity in *Brassica juncea*, suggesting improved light capture efficiency through altered morphological plasticity.

DISCUSSION

The present study demonstrates that LED photoperiod timing significantly influences growth patterns and morphological development in *Brassica juncea*, with results that

Table 5
*Effect of various LED lighting timings on SPAD of mustard greens (*Brassica juncea *L.)*

Treatment	Mean SPAD Index				
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS
LED_0	26.83c	29.14	30.65	31.45b	32.62
LED_18-22 pm	30.07a	29.40	30.89	32.62b	32.14
LED_22 pm-2 am	28.28b	29.67	30.85	31.11b	33.06
LED_2-6 am	27.17c	29.60	30.48	35.83a	33.24
Sig.	***	ns	ns	***	ns

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am, and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: *** ($P < 0.001$), ns (not significant). Days after sowing: DAS

Table 6
*Effect of various LED lighting timings on yield components and the ratio of biomass allocation of mustard greens (*Brassica juncea *L.)*

Treatment	Fresh Weight (g)				Fresh Biomass Allocation (%)		
	Root	Stem	Leaf	Total	Root	Stem	leaf
LED_0	1.24ab	0.26b	11.74ab	13.07b	9.22a	1.92b	90.15a
LED_18-22 pm	1.48a	4.19a	15.68a	20.65a	8.50ab	17.33a	77.06b
LED_22 pm-2 am	0.76bc	2.48a	9.55bc	12.65b	7.75ab	20.94a	77.13b
LED_2-6 am	0.66c	2.55a	6.86c	10.07b	6.54b	21.73a	71.73b
Sig.	**	*	**	**	*	**	***

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am, and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$)

both corroborate and extend previous findings on photoperiod-specific optimisation for Brassica species. The superior performance of the LED_18-22 pm treatment validates the critical importance of timing windows identified in recent literature while revealing novel trade-offs in resource allocation.

Interpreting Growth Responses at Low Supplemental PPFD

The PPFD delivered by the LED modules (60-70 $\mu mol.m^{-2}.s^{-1}$ at 0.5 m) is well below the light-saturation range reported for Brassicaceae under continuous daytime supplementation (100-300 $\mu mol.m^{-2}.s^{-1}$), but matches independent measurements from our own group using the same CoB LED system (47 $\mu mol.m^{-2}.s^{-1}$ in Duong et al. (2024) and 30.50-158.38 $\mu mol.m^{-2}.s^{-1}$ across the canopy in Duong et al. (2026)), confirming this is the true irradiance rather than measurement error.

Given this sub-saturating PPFD, we propose that the pronounced morphological responses - notably the 70.7% increase in plant height under LED_18-22 pm despite modest leaf-dimension gains - are not primarily driven by enhanced photosynthetic carbon assimilation. Since all treatments were delivered as discrete nighttime pulses rather than continuous daytime light, a low-fluence photomorphogenic signal mediated by phytochrome/cryptochrome is more plausible, consistent with classical night-break and photoperiod-extension responses in Brassica species. Under this view, the LED pulses act as a circadian/photoperiod cue - shifting perceived night length and redirecting assimilate partitioning toward stem elongation - rather than as a direct carbon source. This aligns with our companion study (Duong et al., 2024), where exposure timing and duration, not PPFD magnitude, principally determined growth and flowering under similarly low irradiance (47-58 $\mu mol.m^{-2}.s^{-1}$). The decoupling between height enhancement (70.7%) and biomass gain (58.0%) further supports a photomorphogenic rather than purely photosynthetic explanation, since a pure carbon-assimilation effect should scale more proportionally with biomass than elongation.

Photoperiod Duration and Biomass Enhancement

The 70.7% increase in plant height and 58.0% enhancement in total fresh weight under LED_18-22 pm treatment strongly supports (Duong et al., 2024) findings that 4-hour evening LED supplementation (creating 16-hour total photoperiods) optimises mustard green growth parameters. This performance significantly exceeds the negative effects observed with 6-hour supplementation in their study, confirming the existence of an optimal threshold for supplemental lighting duration. Our results align with Liu et al. (2022)'s demonstration that 14-hour photoperiods maximised biomass in cabbage and Chinese kale microgreens, suggesting that 16-hour total duration represents near-optimal conditions for *Brassica juncea*. The consistent growth rates maintained throughout periods

2-4 under LED_18-22 pm treatment contrast with the declining performance observed in longer photoperiod treatments, supporting (Pham et al., 2024) observations that extended LED durations can shift from beneficial to detrimental effects. This temporal stability validates the hypothesis that evening supplementation optimally synchronises with natural circadian rhythms without disrupting essential dark-period processes. Interestingly, our finding that LED_18-22 pm outperformed LED_2-6 am for total biomass contrasts with a recent hydroponic trial using an identical 4-hour treatment structure, in which the pre-dawn (02:00-06:00) window, rather than the evening window, produced the highest fresh and dry biomass along with the greatest leaf-to-stem ratio (Duong et al., 2026). This divergence may reflect fundamental differences between soil-based and hydroponic nutrient delivery, or between the specific mustard cultivars used, and suggests that the optimal LED timing window may not be universally transferable across production systems. Further comparative trials using matched cultivars and growing media are needed to determine whether soil-grown *Brassica juncea* responds differently to nocturnal LED timing than hydroponically grown counterparts.

Light Intensity Effects and Morphological Responses

The enhanced early chlorophyll synthesis under LED_18-22 pm treatment (12.1% increase at 14 DAS) corroborates findings by (Liu et al., 2022) that optimal light intensity promotes higher SPAD values in Brassica species. However, the convergence of chlorophyll levels at harvest suggests that photoperiod timing primarily influences synthesis kinetics rather than final accumulation, extending (Singh et al., 2020) observations on SPAD meter applications for monitoring photosynthetic responses. The pronounced trade-off between vertical growth enhancement and leaf dimension reduction provides new insights into the morphological plasticity described by (Gerovac et al., 2016). While their study showed that higher light intensity generally decreases plant height in microgreens, our results demonstrate that timing-specific supplementation can reverse this relationship, achieving both height enhancement and substantial biomass increases. The 11.8% reduction in leaf width and 18.2% decrease in leaf length under LED_18-22 pm treatment suggest resource reallocation mechanisms similar to those observed under varying light intensities, but with timing-dependent modulation.

Biomass Allocation Patterns and Architectural Changes

The dramatic shift in biomass allocation from 90.15% leaf allocation in controls to 77.06% under LED_18-22 pm treatment, with stem allocation increasing from 1.92% to 17.33%, provides quantitative validation of Pham et al. (2024) observations that LED duration influences biomass partitioning. This reallocation pattern exceeds the magnitude reported in their study, likely due to the specific timing optimisation achieved with evening supplementation. The even more pronounced stem allocation under LED_22 pm-2 am (20.94%) and LED_2-6 am (21.73%) treatments, despite reduced total biomass, suggests that night-time supplementation may trigger different physiological responses than evening extension. These allocation changes align with findings that biomass partitioning shifts from leaves to stems at later developmental stages (Singh et al., 2013), but our results indicate that LED timing can accelerate or modify this natural progression. The maintained photosynthetic capacity despite reduced leaf allocation suggests improved light capture efficiency through architectural modification, supporting the morphological plasticity hypothesis proposed in recent literature.

Chlorophyll Dynamics and Photosynthetic Optimisation

The temporal SPAD responses reveal sophisticated photoperiod-dependent metabolic programming previously unrecognised in Brassica LED studies. The early enhancement under LED_18-22 pm followed by superior mid-season response of LED_2-6 am treatment (13.9% increase at 35 DAS) demonstrates that different timing windows optimise distinct phases of chlorophyll development. This finding extends observations on spectral effects to include temporal optimisation, suggesting that both when and how LED supplementation is applied critically influences photosynthetic capacity (Li et al., 2019; Wu et al., 2013). The convergence of SPAD values at harvest, despite significant mid-season differences, corroborates Andrew (2023) observations that chlorophyll responds dynamically to light environments while maintaining homeostatic regulation. This temporal pattern suggests opportunities for stage-specific LED management strategies that optimise photosynthetic efficiency throughout the growing cycle.

Leaf Development and Production Efficiency

The exceptional leaf production burst under LED_18-22 pm treatment (0.53 leaves·day^{-1} from 14 to 21 after sowing), followed by a dramatic reduction, provides new insights into the timing-dependent responses reported by Duong et al. (2024). This pattern suggests that evening LED supplementation may trigger accelerated developmental programming that front-loads leaf production but potentially limits sustained growth capacity. The contrast with LED_2-6 am treatment's more consistent leaf production (0.39, 0.11, and 0.17 leaves·day^{-1} for periods 2-4) indicates alternative optimisation strategies for different production objectives. These findings extend observations on continuous lighting effects to reveal that timing-specific supplementation can achieve similar yield enhancement with potentially greater energy efficiency (Shibaeva et al., 2022). The 26.2% increase in leaf number under LED_18-22 pm treatment at 35 DAS approaches the yield improvements reported for continuous LED systems while using significantly less energy input.

Integration with Mustard Green Production Systems

The results provide important validation and extension of recent microgreen research. The enhanced fresh weight accumulation under LED_18-22 pm treatment (15.68 g vs. 11.74 g in controls) aligns with Gerovac et al. (2016) findings that appropriate light management increases biomass in mustard greens. However, our study demonstrates that timing optimisation can achieve these benefits while maintaining larger plant architecture, suggesting applications beyond microgreen production. The shorter harvest time implications identified by Duong et al. (2024) are supported by our growth rate data, particularly the accelerated early development under evening LED supplementation. This acceleration, combined with the maintained quality parameters, suggests commercial potential for LED timing optimisation in various Brassica production systems.

Implications for Controlled Environment Agriculture

The study's findings have significant implications for precision lighting strategies in controlled environment agriculture. The optimal 4-hour evening supplementation window identified validates recent industry trends toward targeted photoperiod management rather than continuous supplementation. The substantial yield improvements (58.0% increase in total biomass) achieved with relatively modest energy inputs support the economic viability of timing-specific LED strategies. However, the observed trade-offs between height enhancement and leaf expansion suggest that production objectives must guide LED timing selection. For applications prioritising leaf quality and surface area, alternative timing strategies or hybrid approaches may be more appropriate than the optimal evening supplementation identified for biomass production.

Future Research Directions and Limitations

While this study provides crucial insights into timing-specific LED responses, several areas warrant further investigation. The incomplete data for LED_22pm-2 am and LED_2-6 am treatments in leaf morphology parameters limit comprehensive comparison of night-time vs. early morning supplementation strategies. Future research should investigate the molecular mechanisms underlying these timing-dependent responses, particularly circadian clock gene expression and photosynthetic enzyme regulation patterns. The study's focus on morphological and yield parameters should be extended to include nutritional quality assessments, as recent literature suggests that LED timing may influence secondary metabolite production and antioxidant capacity. Based on the LED specifications (50 W/module), total installed power was ~150 W per treatment (3 modules), giving ~0.6 kWh/day (4-hour photoperiod) and ~21 kWh per treatment over the lighting period, or ~63 kWh across all three LED treatments. A full cost-benefit analysis was not performed, as this depends on fluctuating vegetable prices and additional capital costs beyond this study's scope.

However, prospects remain promising given improving LED efficiency and the growing use of solar power as a lower-cost, lower-emission alternative to fossil-fuel electricity in tropical greenhouse lighting. Additionally, longer-term evaluations could reveal whether the observed architectural changes translate into sustained productivity advantages under commercial growing conditions. The findings establish LED timing as a critical optimisation parameter for *Brassica juncea* production, with evening supplementation (18-22 pm) providing superior yield enhancement while revealing important morphological trade-offs that must be considered in precision agriculture applications.

CONCLUSION

This study demonstrates that LED photoperiod timing is critical for optimising *Brassica juncea* growth, with evening supplementation (18-22 pm) providing superior results. The LED_18-22 pm treatment achieved remarkable enhancements of 70.7% in plant height, 58.0% in total fresh weight, and 26.2% in leaf number compared to controls. However, this optimisation involved significant morphological trade-offs, including reduced leaf width (11.8%) and length (18.2%), with biomass allocation shifting dramatically from leaves (90.15% to 77.06%) to stems (1.92% to 17.33%). The temporal chlorophyll responses revealed stage-specific optimisation opportunities, with evening supplementation enhancing early development and early morning treatment improving mid-season chlorophyll content. These findings validate that 4-hour evening LED supplementation represents an energy-efficient strategy for maximising mustard green productivity while highlighting the importance of considering morphological consequences in commercial applications. The research establishes timing-specific LED protocols as essential tools for precision agriculture, demonstrating that when supplementation is applied is as critical as how it is applied for achieving optimal crop production outcomes. From a practical standpoint, the 4-hour evening LED supplementation (18-22 hours) required only a modest amount of electricity (~21 kWh per treatment over the growing period) while delivering a substantial yield gain (58%), indicating that this strategy is feasible for implementation in greenhouse or indoor production systems. In tropical regions with abundant solar radiation, pairing the LED system with solar power could further reduce operating costs and improve environmental sustainability, a direction that warrants dedicated evaluation in future studies.

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